LIFETIME PCB 153 BIOACCUMULATION AND PHARMACOKINETICS IN PILOT WHALES: BAYESIAN POPULATION PBPK MODELING AND MARKOV CHAIN MONTE CARLO SIMULATIONS

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Introduction

Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the uptake, distribution and elimination processes of chemicals in organisms integrating physiology, biochemistry, pharmacology and toxicology of the chemicals of interest[1]. Such models were originally used to describe and predict the kinetics of drugs in rodents and humans, but have gained importance in environmental toxicology in recent years[1]. The experiments involved in PBPK modeling can be minimally- or non-invasive; thus, this approach is of particular interest for assessing pollution in wild marine mammals since all toxicological experiments are prohibited or ethically undesirable in these animals. Marine mammals typically accumulate considerable amounts of chemicals in their tissues because of their long life spans and their top position in the aquatic food webs[2]. So far, PBPK models for chemicals in marine mammals have been parameterized by adopting those from other organisms and validated using results measured in tissue samples from dead, stranded animals[3,4]. However, such an approach entails a fair amount of model uncertainty. Bayesian methods and Markov chain Monte Carlo (MCMC) simulations are currently the best statistical approaches to address this uncertainty[5]. This method results in posterior probability distributions in which the parameter values are much more robust for the species and the chemicals of interest[5, 19-20].

The goal of the present study was therefore to develop a PBPK model for PCB 153, one of the most persistent pollutants in marine mammals, in pilot whales and to evaluate that model using the Bayesian approach and MCMC simulations.

Materials and methods

The development of the PBPK model for lifetime exposure to PCB 153 in male pilot whales can be separated into two phases: the development of the structural PBPK model and the evaluation of its parameters using the Bayesian approach and MCMC in a statistical model.

- *Structural PBPK model.* Since only blubber samples were available, the structural PBPK model for lifetime accumulation of PCB 153 in male pilot whales consisted of only two compartments, namely the blubber and a compartment that accounts for the rest of the body. In this way, the blubber compartment could be kept as a storage compartment for lipophilic compounds, whereas intake and elimination processes (metabolic biotransformation and excretion) were set in the 'rest of the body'-compartment. All compartments were considered to be flow-limited, similar to the PBPK models for lifetime accumulation of PCB 153 in harbour porpoises[4]. The uptake of PCB 153 occurs in marine mammals mainly through milk and food intake. Long-finned pilot whale (*Globicephala melas*) calves can drink milk from their mothers for up to 3 years, but switch gradually to a fish/cephalopod diet after one year of suckling. However, for modeling purposes, the animals were assumed to drink only milk during their first year of life after which a fish/cephalopod diet was taken as the only source for intake. For both fish/cephalopod and milk, an assimilation efficiency of 90% was used[3]. The daily intake for the milk diet was set at 4 kg[6], whereas the daily intake for the fish/cephalopod diet was body weight-dependent[7]. *In vitro* studies with pilot whale hepatic cells have, to our knowledge, never been performed; thus, we have no knowledge regarding their metabolic capability. Furthermore, pilot whale feces and urine samples

were not available. Therefore, all these elimination pathways were lumped together, and a clearance constant was derived from an estimated elimination half-life of 27.5 years for PCB 153 in humans and harbour porpoises[4,8]. The growth dilution effect was taken into account by incorporating the growth equations from Bloch et al.[9] into the model. An equation for the body weight dependent cardiac output together with the portions of the blood flow going to each compartment were taken from Altman and Dittmer[10] and from Brown et al.[11], respectively. The proportion of blubber in long-finned pilot whales was set at 25% of body weight[12]. A lipid percentage of 84.4 % for blubber was used to express the final outcome of the model in PCB153 concentration per unit lipid weight (lw)[13]. The lipid percentage for the 'rest of the body'-compartment was 5.2 % and was the average of the lipid percentages of liver, kidney, brain and muscle of male harbour porpoises[4]. Input concentrations, being the levels of PCB 153 in milk and fish/cephalopods, were not available. However, since these input parameters typically have a high impact on the model outcome, they were automatically included in the statistical model and estimated through MCMC simulations (Table 1).

- *Statistical model.* A Bayesian approach, implemented using Markov chain Monte Carlo (MCMC) analysis, was applied in order to evaluate or update the parameters taken from the literature (priors) with regard to the current pilot whale data. In the present study, the priors were taken from the literature or were deduced from the harbour porpoise model[4]. The entire structural model contained more than 15 different parameters, however, not all parameters were included in the statistical model for MCMC analyses. Parameters with the highest influence on the PBPK model outcome were selected by performing a global sensitivity analysis (GSA) first. The prior parameter values for these selected parameters together with their posterior probability distribution, characterized by a mean and standard deviation, are shown in Table 1. In order to allow the curves to converge and to be able to calculate convergence factors (R-values[21]), three chains of 5,000 iterations each were used. The development of the PBPK model, the GSA and all simulations were performed using AcslX/Libero software (AEgis Technologies, Orlando, FL). Simultaneously, we are also building such a PBPK model using MCSim for comparison[22].

- *Pilot whale data*. PCB 153 results in blubber samples from 21 male pilot whales and one fetus were used. Body sizes were recorded for all animals, except for the fetus. The age of the animals could not be assessed through counting dentine layers, but was estimated via the recorded body size of each animal and the growth equations for long-finned pilot whales from Bloch et al.[9]. All animals were victims of the mass-stranding at Sandy Cape, Tasmania, Australia on December 14, 2008. In all samples, 37 PCB congeners, 6 PBDEs, 6 DDXs, HCB, chlordanes (CHLs) and 5 MeO-PBDEs were targeted of which only the PCB 153 results were used in the present study. Information about the sample preparation and GC-MS analyses were described previously[13].

Results and discussion

This PBPK model for bioaccumulation and pharmacokinetics of PCB 153 in male pilot whales should be seen as a preliminary model for two reasons. First, the model has the potential to be transformed into a multi-compartment model as soon as information about levels of PCBs in tissues other than blubber becomes available. Second, the Bayesian approach allows progressive updates of the current model with new datasets. Nevertheless, despite its preliminary nature, the model in this study is an interesting starting point to evaluate the PCB 153 levels analyzed in blubber of male pilot whales and provides new ideas for future studies to focus on.

PCB 153 levels in male pilot whales

Figure 1 shows the concentration of PCB 153 in blubber of male pilot whales at different ages from Tasmania. There is a steep increase in PCB 153 level from birth until the age of approximately 1.5 years followed by a rapid decline until the age of about 3 years. A similar pattern was also found for the PCB 153 concentrations in harbour porpoises[4]. In that case, the steep increase and rapid decline were mostly due to the large difference in PCB 153 concentrations in the milk diet and fish diet of the harbour porpoises. However, the PCB 153 level in the milk (CMILK, Table 1) of pilot whales appears to be much lower than the concentration in the fish/cephalopod diet (TOTDIET, Table 1). In the current study, the input of milk in the pilot whales was calculated from the concentration in the milk multiplied by the daily consumption of milk and the assimilation efficiency. Milk samples of pilot whales were not available in this study or in the literature which is the reason why this parameter was included in the MCMC simulations. The daily consumption of milk of 4 kg of milk/day, on the other hand, was taken from Oftedal[6] and was in that study estimated based on the mass of the mammary gland and the growth rate of the suckling animals. As the mothers can nurse more offspring at the same time, the

amount of milk used in the current study is probably much higher than any calf receives in reality. In addition, the assimilation efficiency was set as 90%, similar to that for the harbour porpoises[4] which is in general fairly high. Overestimations of either the daily consumption of milk or the assimilation efficiency from milk automatically lead to underestimations of the concentrations of PCB 153 in milk. The resulting relatively low level for the PCB 153 level in milk is therefore a preliminary conclusion with regard to the current model set-up and available information. Male pilot whales grow fast from birth to adulthood (around 10 years of age) and this dilution effect of PCB 153 in tissues due to body mass increase definitely plays a role in the initial phase (Figure 1) of the overall decrease of PCB 153 levels in blubber. However, as the growth levels off after that age, the slight decline in PCB 153 concentrations at higher ages might have been due to other factors.

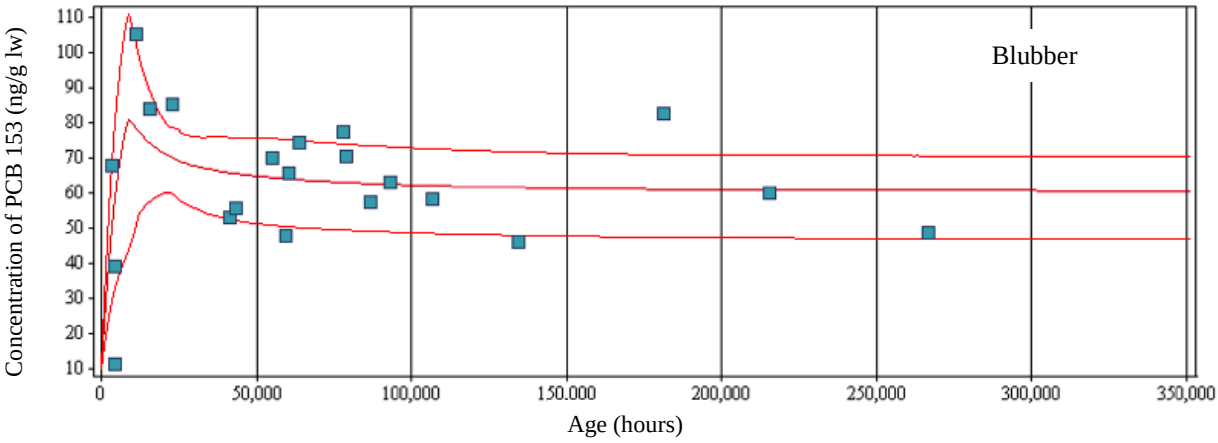

Figure 1. Concentration of PCB 153 (expressed in ng/g lw) with age in blubber of male pilot whales from Tasmania. ___ = curves for mean ± 3σ with 3σ including about 99.7% of the population based on the marginal priors, ■ = individual data-points of male pilot whales (n = 21), X-axis goes to 350,400 hours which is 40 years.

Modeling lipophilic compounds in pilot whales

Understanding the kinetics of compounds in pilot whales, or marine mammals, in general, is a challenging task. Their protected status does not allow real-life exposure experiments; thus, parameters from typical organisms such as rodents are often used in marine mammal PBPK modeling work[4]. Consequently, the PBPK model is usually capable of explaining only a part of the marine mammal dataset. The method applied in the present study starts with estimated or known parameters values from the literature and uses the entire available dataset to come up with new parameter values. These new parameter values can accordingly be used further as "priors" as soon as new datasets are available resulting in more updated parameter values. Such a Bayesian approach would provide progressively more robust results so that the updated parameters are better applicable for the population bioaccumulation model for wild animals.

Table 1. Prior parameter values, posterior probability distributions and R-values (convergence factors) of the parameters that were updated in the Bayesian PBPK modeling and MCMC simulations.

	Prior			Posterior		
Parameter	Value	Range	Distribution	Mean	StD	R-value
PF	331.6*	0 - 400	normal	326.7	45.3	1.0124
PR	7.9$	0 - 40	normal	8.7	3.3	1.0020
TOTDIET	0.3	$1x10^{-10}$ - 1	normal	0.290	0.041	1.0146
CMILK	0.006	$1x10^{-10}$ - 1	normal	$4.8x10^{-3}$	$8.2x10^{-4}$	1.0083

PF-Blood/fat partition coefficient (no unit), PR-Blood/rest of the body partition coefficient (no unit), TOTDIET-concentration of PCB 153 in the fish/cephalopods diet of pilot whales (ng/g ww), CMILK-concentration of PCB 153 in the milk of pilot whales (ng/g ww), *-based on Weijs et al.[4] and Parham et al.[14], $-average of PL, PB, PK and PR from Weijs et al.[4]

The present study has taken parameter values from the literature (priors) and has used the PCB 153 data measured in male pilot whales from Tasmania to update those values (posteriors) (Table 1). Of course, with each new available dataset, the parameter values can be further evaluated and updated. From this perspective, the present study is the first step towards the production of reliable parameter values for pilot whales. However,

even though the posterior parameter values in this study are by no means final, they are more credible for pilot whales than any existing information so far.

Future Perspective

In recent years, multiple studies have been conducted on the influence of POPs on the development of obesity in humans. POPs, such as dioxins and PCBs, have the potential to differentiate adipocytes, to alter fat levels in the blood and to elevate triglyceride levels[15,16]. *Odontocetes* use certain lipid deposits (melon and lower jaw fat) to echolocate. In these lipid structures, the lipid composition has to remain the same in order to maintain the echolocation capacity. Yordy et al.[17] reported high concentrations of PCBs and PBDEs in the melons of bottlenose dolphins and suggested that the melons can serve as an alternate depot for POPs coming from the blubber. Additionally, it has been found that concentrations of some lipid classes in the melon vary with the age of the animals[18]. From these studies, it seems that there is continuous transport of lipids (and associated lipophilic compounds) towards the melon. As a consequence, some scientists are interested in finding out whether the concentrations of lipophilic compounds in the body of *odontocetes* have an impact on the organization of lipids in the melon and therefore on their capacity for echolocation. Taking samples from the melon is, unlike blood or blubber samples, a destructive procedure and therefore prohibited in live marine mammals. Although there is a long way to go between investigating lipophilic compounds, lipids and echolocation, PBPK models, such as the one presented here, would be capable of showing the fate of pollutants in the melon compared to their kinetics in the rest of the body. At the beginning, this should be done using tissue samples from dead, stranded animals. Once the models have been developed and parameters have been established using several different datasets, concentrations in the melon can be predicted with the models and blubber samples only. Since blubber samples can be obtained in a minimally-invasive manner through dart-biopsy sampling, this approach would open the door towards echolocation studies.

Acknowledgements

This paper is dedicated to Tony (AC) Roach who initiated this study, but passed away unexpectedly in May 2011. LW is financially supported by a PhD scholarship from the Scientific Research Foundation (FWO) in Belgium. The present study was funded by a VOCATIO grant awarded to LW; partial funding by the Australian Department of Sustainability, Environment, Water, Population and Communities, under the Chemical Monitoring Initiative, is also acknowledged. Assistance from Dr Karrie Rose (Zoological Parks Board of NSW, Taronga Conservation Society Australia) and from Kris Carlyon, Drew Lee and Rupert Davies from DPIPWE, Tasmania, for transporting and sourcing the samples is appreciated.

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